REMARKS

Reconsideration of the rejections in the Office Action dated July 30, 2001 is respectfully requested.

Applicants petition the Commissioner for a 3-month extension of time. A separate petition accompanies this amendment.

Also attached is a separate page entitled "Version with markings to show changes made" showing the requested changes.

I. Amendments

Claims 1 and 4-7 are presently pending in the subject application. Claims 8-14 have been cancelled in accordance with the restriction requirement imposed by the Examiner; and claims 1 and 4-7 have been amended.

A. Amendments to the Specification

As suggested by the Examiner, the specification has been amended at page 1, line 7 to refer to the filing date of U.S. provisional patent application no. 60/112,324 to which the present application claims priority. The specification has been amended at page 3, line 36; page 7, line 33; page 8, lines 8-9; page 14, lines 12-13; page 14, line 42; and page 17, lines 15 and 17 to list the appropriate SEQ ID NOS for sequences disclosed in the specification. The specification has been amended at page 4, line 6 to correct the reference to Figures 5A and 5B. No new matter to the specification has been added by these amendments. The remaining amendments to the specification consist of correction of typographical or obvious errors and do not constitute new matter.

B. Amendments to the Claims

Claim 1 has been amended to specify that antigen is linked to an added peptidic sequence which may be selected from any one of SEQ ID NO: 1-9. Support for this amendment to claim 1 can be found in the specification at page 7, line 16 to page 8, line 36 and in the Examples. Claims 2 and 3 have been cancelled. Claim 5 has been amended to

correct a typographical error. Claims 4, 6 and 7 have been amended to provide correct the dependency of the claims in light of the cancellation of claim 2. Claims 7-14 have been cancelled in accordance with Applicants's election of Group I for prosecution in the present application.

No new matter has been added by these claim amendments.

II. Rejections under 35 U.S.C. § 112

Written Description

The Office has rejected claim 1 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention. In particular, the Office asserts that the specification does not provide an adequate written description of the "added peptide sequence."[1] Applicants respectfully traverse and request that this rejection be withdrawn in light of the amendment to claim 1.

As amended, claim 1 is directed to an antigenic composition which capable of eliciting an enhanced CTL response in the context of a MHC class I molecule, comprising an antigen having an added peptidic sequence comprising one or more sequences selected from the group consisting of SEQ ID NOS 1-9 which facilitates entry of said antigen into APCs. As noted in the Office action, antigenic compositions comprising an antigen conjugated to one or more peptidic sequences selected from SEQ ID NO: 1-9 are disclosed and adequately supported in the specification.[2] As such, Applicants respectfully submit that one skilled in the art would recognize that the inventors were in possession of the composition of claim 1 and request that this basis for rejection be withdrawn.

Enablement

The Office has rejected claim 1as containing subject matter which is not described in the specification in such a way to enable one skilled in the art to practice the invention commensurate in scope with the claims.[3]

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1 *See* Office action at pages 3-4.

2 *See* Office action at page 4; Specification at page 7, line 16 to page 8, line 36; Examples.

3 *See* Office action at pages 5-6.

As amended, claim 1 is directed to a composition comprising an antigen conjugated to an additional peptidic sequence comprising one or more sequences selected from the group consisting of SEQ ID NO: 1-9 which facilitates entry of said antigen into antigen presenting cells. The Office has noted that an antigenic composition comprising an antigen and a peptidic sequence that comprises one or more of SEQ ID NOS 1-9 are enabled by the present specification.[4] Accordingly, Applicants respectfully request the withdrawal of the rejection of claim 1, as amended.

III. Rejections under 35 U.S.C. §103(a)

The Office has rejected claims 1, 2 and 4-7 under 35 U.S.C. §103(a) as being unpatentable over Buschle et al. (*PNAS USA* 94: 3256-3261, 1997) in view of Kim et al. (*J. Immunol.* 159(4): 1666-68, 1997). Claim 2 has been cancelled so this rejection with regard to claim 2 is moot. For the reasons set out herein, Applicants traverse this Section 103 rejection of claims 1 and 4-7.

As amended, claim 1 is directed to a composition comprising an antigen conjugated to an additional peptidic sequence comprising one or more sequences selected from the group consisting of SEQ ID NO: 1-9 which facilitates entry of said antigen into antigen presenting cells. Buschle et al. disclose that polyarginine (pArg) and polylysine (pLys) enhance the uptake of peptides by APCs. Specifically, bone marrow-derived APCs were incubated with a peptide alone or a combination of labelled peptide plus pLys or pArg, and the amount of peptide transported into the APC was measured.[5] As noted in the Office action, Buschle et al. do not disclose a composition comprising an antigen and an added peptidic sequence and Buschle et al. neither disclose, teach or suggest that the added peptidic sequence is linked to said antigen nor that the peptidic antigen-polycationic

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[4] *See* Office action at page 5.

[5] *See* page 3258-9 of Buschle et al.

sequence is a fusion protein. In particular, Buschle et al. do not disclose, teach or suggest that the cationic peptide is one or more of the sequences selected from SEQ ID NO: 1-9. Kim et al. disclose exposing antigen presenting cells to a composition containing a soluble protein conjugated to a short cationic peptide derived from HIV-*tat*. Kim et al., however, do not disclose a composition which comprises an antigen covalently conjugated to an added peptidic sequence comprising one or more sequences selected from SEQ ID NOS: 1-9.

Applicants urge that one skilled in the art would not have been motivated by the disclosure in either Buschle et al. or Kim et al. to design a composition comprising an antigen and an added peptidic sequence comprising one or more sequences selected from SEQ ID NOS: 1-9 as claimed in amended claim 1of the present application. Buschle et al. do not disclose or suggest the use of pArg, pLys or other cationic peptides to aid in the entry of peptides into APC for the purposes of elicting an enchanced cytotoxic T cell response in the context of a major histocompatiability complex class I molecule. Rather, the focus of the study conducted by Buschle et al. is the ability of various polycationic peptides to aid in the transport of proteins into cells. Importantly, Buschle et al. do not disclose, teach or suggest that the cationic peptide is one or more of the sequences selected from SEQ ID NO: 1-9. This lack of disclosure in Buschle et al. is not compensated by the disclosure of Kim et al. Kim et al. disclose OVA conjugated to a cationic peptide derived from HIV-*tat*. Like Buschle et al., Kim et al. do not disclose or suggest that the sequence of the peptide to which the antigen is conjugated is one or more sequences selected from SEQ ID NOS: 1-9. As such, one skilled in the art would not arrive at the composition of amended claim 1. For these reasons, Applicants respectfully submit that amended claim 1 is not obvious over Buschle et al. in light of Kim et al. and request that this rejection be withdrawn.

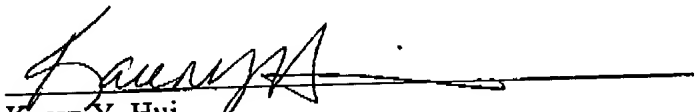
Claims 4-7 are dependent upon claim 1 and are not obvious over Buschle et al. in light of Kim et al. for the same reasons stated with respect to claim 1. As such, Applicants respectfully request that this rejection be withdrawn with respect to claims 4-7.

IV. CONCLUSION

In light of the foregoing amendments and remarks, pending claims 1 and 4-7 are in condition for allowance. Applicants, therefore, request reconsideration of the application and an allowance of all pending claims. If the Examiner wishes to discuss the above-noted distinctions between the claims and the cited references, or any other distinctions, the Examiner is encouraged to contact the undersigned by telephone. Additionally, if the Examiner notices any informalities in the claims, he is also encouraged to contact the undersigned to expediently correct any such informalities.

Respectfully submitted,

Perkins Coie LLP



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Appendix (Marked-up version of claims)

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

This application claims priority to U.S. Provisional application Serial No. 60/112,324, **filed December 14, 1998,** expressly incorporated by reference herein.

Figure 3 shows the response of B3Z T cell hybridomas to APC prepulsed with SIINFEKL **(SEQ ID NO: 10)** or OVA or OVA-pEA/pK at their respective optimal concentrations. Cpm corresponds to cell growth as described above for Figs. 2A-C.

Figures 5A-B show the survival of E.G7-OVA injected mice treated with Ag-pulsed APC. Twenty 8-week old randomized female C57BL/6 mice were injected i.p. with 25×10^6 and 2×10^6 E.G7-OVA cells in 0.1 ml PBS (Fig. **[6]5**A and Fig. **[6]5**B, respectively). Two days and again 2 weeks later (arrows), mice received i.p. injections of DC, OVA-pulsed DC, OVA-pEA/pK-pulsed DC (5×10^5 cells per 0.1 ml injection), or PBS. Mice were monitored daily and their survival was recorded as indicated.

In another preferred embodiment, the peptidic sequence comprises repeating subunits having about 6 amino acids per subunit wherein a given **[sequences] sequence** has 3 or more of such subunits and may or may not have an added N-terminal cysteine. Exemplary peptides are presented as CYS-[X-Y-Y-Y-Y-Y]$_n$ **(SEQ ID NO: 8)**; wherein X= glu or asp,. Y= ala, leu, ile, phe, gly, cys, met or val and n is greater than or equal to 3 **[(designated sequence 8)]**, with a specific example provided by the pEA peptide presented as SEQ ID NO: 2.

In another preferred embodiment, the present invention provides an antigen composition for *in vivo* administration comprising one or more soluble protein antigens covalently conjugated to peptides selected from the group consisting of the pK, pEA, HA, tandem pEA/pK, tandem HA/pK peptides, peptides comprising lysine and arginine residues and peptides comprsing repeating subunits presented as SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, **[the sequences**

designated sequence number 8 and sequence number 9, respectively (Table 1)] **SEQ ID NO: 8 and SEQ ID NO: 9**.

The modified soluble **[proteins]** **protein** antigens of the invention whether produced by chemical coupling or by expression of **[continuos]** **continuous** coding sequences as recombinant fusion proteins may be used to pulse APC, and be presented as such APC in the context of MHC I.

In a related aspect, the cancer-specific or tumor antigen is modified by a covalent conjunction to a peptide selected from the group consiting of the pK, pEA, HA, tandem pEA.PK, tandem HA/pK peptides, peptides comprising repeating units presented as SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 **[and the sequences designated as sequence number 8 and sequence number 9, respectively]**, **SEQ ID NO: 8 and SEQ ID NO: 9**.

The IL-2 secreting mouse T cell hybridoma B3Z, which responds to the mouse MHC class I ($H2\text{-}K^b$) bound OVA-derived peptide SIINFEKL **(SEQ ID NO: 10)** ($OVA_{257\text{-}264}$; Jameson *et al.*, *J. Exp. Med.* 177: 1541, 1993), was used to evaluate the presentation efficacy of various peptide conjugates by the thymoma cell line EL-4.

The above evidence appears to reflect improved penetration of pEA/pK-conjugated OCA into the class I-dependent Ag processing and presentation pathway. An additional experiment was conducted in order to eliminate the possibility that Ag-derived peptides in the conjugate (which do not require internalization or processing), were responsible for the observed improvement in B3Z responses. Cells were cultured as described above and EL-4 cells were fixed with 0.025% glutaraldehyde (Fluka, Buchs, Switzerland) prior to Ag pulsing. When APC were fixed with glutaraldehyde prior to Ag pulsing thereby preventing Ag internalization and processing, fixed EL-4 cells were observed to present the immunogenic peptide SIINFEKL **(SEQ ID NO: 10)** to B3Z in a stimulatory fashion, while their capability to present OVA-pEA/pK was completely lost upon fixation (see Fig. 3). SIINFEKL **(SEQ ID NO: 10)** is an OVA-derived peptide

($OVA_{257-264}$), recognized by the T cell hybridoma B3Z. (Jameson *et al.*, 1993, J. Exp. Med. 177: 1541) A residual (approximately 7%) B3Z response to fixed EL-4 pulsed with unmodified OVA was observed indicating that the latter, though 99% pure (Sigma, St. Louis, MO) may contain a minor fraction of degradation product(s), which are removed upon conjugation to pEA/pK.

In the Claims :

1. (amended) An antigen composition capable of eliciting an enhanced cytotoxic T cell response in the context of a major histocompatibility complex class I molecule (MHC class I), comprising an antigen having an added peptidic sequence **comprising one or more sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, wherein said added peptidic sequence** facilitates entry of said antigen into antigen presenting cells (APC).

4. (amended) The antigen compositon of claim **[2] 1**, wherein said antigen is a soluble protein antigen.

5. (amended) The antigen composition of claim 4 for use in immunizing a subject against a tumor or pathogen wherein said **[anitgen] antigen** is specific to the tumor or pathogen.

6. (amended) The antigen composition of claim **[2] 1**, wherein said one or more added peptidic sequences are covalently linked to said antigen.

7. (amended) The antigen composition of claim **[2] 1** wherein said antigen is a fusion protein produced by translation of a continuous nucleotide coding sequence.